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With amended claims.

(54) Title: A METHOD OF INHIBITING ENDOTOXIN INDUCED EFFECTS

(I)

(57) Abstract

The invention relates to the use of a compound according to formula (I) in which x is an integer of from 2 to 5 and A signifies -NH-C(=NH)-NH_2,-CH_2-NH_2 or -CO-NH_2 or agmatin, for the preparation of a medicament for treatment of endotoxin induced effects. The preparation is intended to be infused in an amount which corresponds to from 10 to 800, preferably from 10 to 400 mg per kilogram of body weight and hour. The invention also relates to a method for the treatment of an endotoxininduced fever, in which the compound or agmatin are administered in amounts given above, and also to a method for removing endotoxins from solutions in vitro, and to a method of enriching endotoxins.

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A Method of Inhibiting Endotoxin Induced Effects

When manufacturing pharmaceuticals for parenteral use, one of the most important prerequisites is that the products included in the pharmaceutical are non-pyrogenic, i.e. that the endotoxin concentration of the pharmaceutical concerned is so low that only very small biological effects or no biological effects can be detected with conventional test systems (limulus tests = LAL or temperature increase in rabbits). Endotoxins are high molecular complexes associated with the outer cell wall of Gram-negative bacteria (e.g. E. Coli, Proteus or Salmonella), from which lipopolysaccharides (LPS) can be released (endotoxins, O-antigens) (Rietschel, E.T., et al, in Bacterial Endotoxins: Structure, Biomedical Significance and Detection with Limulus Amebocyte Lysate Test, pages 31-50, Alan R. Liss Inc., 1985).

Endotoxins are present in and are often the cause of the clinical symptoms in sepsis and in ARDS and DIC (adult respiratory distress syndrome and direct intravascular coagulation respectively) (Zaren, B. and Hedstrand, U., Intensivvård, pages 63-64, Uppsala University, Reprocentralen HSC, 1989).

Subsequent to having treated patients suffering from, e.g., septicemia with antibiotics, it is well known that the temperature of the patient will rise or that a further fever peak will occur, so-called Herxheimer's reaction, wherewith dead bacteria and parts thereof, including endotoxins, enter the blood circulation.

Clinical signs of the effect of endotoxins (the limit at which these can be shown is about 5 EU per kilo of body weight in rabbits and human beings) can sometimes be

observed when pharmaceuticals and nutrient solutions are administered parenterally. In the case of human beings and rabbits for instance, the clinical signs are manifested by a feverish state, due to the ability of the endotoxins to release endogenic pyrogens which influence the thermoregulatory centre in the central nervous system. Other manifestations can also be observed in the central nervous system (Nowotny, B., Naturwissenschaft 58, pages 397-409, 1971). Such cardiovascular changes as hypotension and permeability changes in arteriole and venules, for instance, may explain certain important organ changes which often occur in Gram-negative sepsis (Zaren, B. and Hedstrand, U., Intensivvård, pages 63-64; Uppsala University, Reprocentralen HSC, 1989; Nowotny, B., Naturwissenschaft 58, pages 397-409, 1971; Gilbert, R.P., Physiol. Rev. 40, 245, 1960; Vick, J.A., Am. J. Phys. 200, 944, 1964).

Those depyrogenizing methods which can be applied in vitro today are based on two principle techniques, namely a) to guard against endotoxin contamination and b) to remove endotoxins during formulation.

It is difficult to carry out the first method a) strictly, because it is necessary for aseptic conditions to prevail during the whole of the formulating process and also during the preparation of starting materials. The second method b) has resulted in the development of different filtering methods, these methods including the use of asbestos filters, ion exchangers, and have involved adsorption on activated carbon or on barium sulphate suspensions, gamma radiation, filtration through membranes having an exclusion limit ranging from 100,000 Daltons to 0.1 micron of endotoxin aggregate, the supply of amebocytlysate and the removal of the gel formed, and also the use of ultrafilters having an

exclusion limit of 10,000 Daltons for filtering-out non-aggregated endotoxins. At the present time, ultrafiltration is primarily applied industrially, whereas the other methods have been abandoned, with the exception of asbestos filtration. Two depyrogenizing methods, namely autoclaving alone or in combination with extremely low pH-values now have limited value because of their low efficiency and because of damage caused to the products (Mosier, L.D., et al. J. Parent. Sci. and Technol., Vol. 41, No. 1, pages 21-25, 1987). The ultrafiltration method, however, results in high production costs, because of the expensive material and high working costs involved. Furthermore, the equipment used is often highly space-consuming and often of doubtful efficiency, resulting in floating exclusion limits and enabling endotoxins to pass through the filters to some extent.

One particular problem in this regard is the assaying of endotoxins in biologically active substances, such as coagulation factor 2 (prothrombin) for instance, or when the sample material is highly restricted but has a very high biological potency, there excluding the use of both the limulus test and experimental animals.

So-called plasmapheresis and hemoperfusion through filters that contain an immobilized product of polymyxin B have been tested in vivo for the purpose of removing endotoxins from the blood path.

Methyl arginine is used as a competitive inhibitor of the ribosylation of ADP, which is necessary in order for endotoxins and cholera toxins to take effect and instigate diarrhea (Moss. J., Garrisson, S., Oppenheimer, N.J., Richardsson, S.H., J. Biol. Chem., Vol. 254, No. 14, pages 6270-6272, 1979).

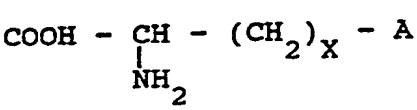
In the case of liver diseases caused by trauma, shock or surgery, it is stated in European Patent Specification No. EP 0059775 that a nutrient solution which contains, inter alia, L-arginine, malic acid, malate, L-asparaginic acid, glucose and carnitine has a protective effect on the liver as a result of stimulating the citrate and urea cycles and therewith lowering the ammonium ion concentrations and phenol concentrations in serum.

Swedish Patent Application No. 8009103-6 teaches a method of increasing the specificity and therewith the effect of corticosteroids, by combining these with esters of, for instance, methyl arginine or ethyl arginine and therewith obtain a synergistic effect. Arginine esters were used in quantities of up to 6.3 mg per kilo of body weight in experiments on rats.

Swedish Patent Application No. 8009102-8 proposes the use of arginine esters as a medicament against endotoxin induced pulmonary oedema. This clinical picture is highly similar to the ARDS condition earlier mentioned. The Claims of this application recite methyl arginine dosages of from 0.25 mg up to 100 mg/kilogram of rat body weight.

Description of the Invention

The invention relates to the use of a compound according to formula I



(I)

in which X is an integer of from 2 to 5 and

A signifies - NH - (C=NH) - NH_2, - CH_2 - NH_2 or - CO - NH_2

or agmatine, for the preparation of a medicament for treating endotoxin-induced effects, particularly for suppressing endotoxin-induced pyrexia.

The medicament is preferably administered orally, intravenously, intramuscularly, intracutaneously or intraperitoneally in an amount of 10-800 mg/kg body weight and hour.

The invention also relates to a method of removing endotoxins from pharmaceutically useful solutions, pharmaceutical preparations, plasma or blood, said method comprising filtering the pharmaceutically useful solution, the pharmaceutical preparation, plasma or blood through a bed which contains an immobilized compound according to formula (I) or immobilized agmatine.

The pharmaceutical preparation may contain a biologically active component, such as a coagulation factor for instance.

The invention also includes the use of an immobilized compound according to formula (I) or the use of immobilized agmatine for removing endotoxins from pharmaceutically useful solutions, pharmaceutical preparations, plasma or blood, and also a method of enriching endotoxins, said method comprising passing a solution containing endotoxins through a bed which contains an immobilized compound according to formula (I) or an immobilized agmatine.

By "arginine or structurally related substances" is meant in the following compounds according to formula (I) or agmatine.
Other features of the invention will be apparent from the following description and from the claims.

The use of the inventive compounds will now be exemplified with the aid of a number of test examples, although it will be understood that these examples do not limit the scope of the invention.

Example 1

5 ng/ml of endotoxins from E. Coli (corresponding to 25 EU/ml endotoxins) together with various amino acids were injected into three live rabbits. The pyrogen reaction was assayed by recording the rectal temperature of the rabbits. The sum of the temperature increases is recited in Table 1. (One to four such experiments were carried out with each amino acid). The result shows clearly that arginine does not result in an increase in temperature of the animals, as distinct from the other amino acids used in the test series (Table 1).

Example 2

When administering a constant infusion of arginine solution (1600 mg/kg and hour) together with ornithine chloride solution (1000 mg/kg and hour) to 8 and 5 rabbits respectively over a period of about six hours, preceded by a bolus dosage of endotoxin (500 EU per kilo body weight), it was noted that the temperature development of these animals was significantly lower than the temperature development of two reference groups (6 and 5 rabbits respectively), which in addition to a bolus dosage of endotoxins corresponding to 500 EU per kilo

body weight were also constant infused with physiological sodium chloride solution (0.9%), and 5% glycose solution respectively over a period of about six hours (see Figure 1 and Table 2).

Table 2

The change in temperature of the rabbits 3.5 hours after injecting endotoxins in an amount corresponding to 500 EU/kg body weight and subsequent constant infusion corresponding to 20 ml/kg body weight of arginine, ornithine chloride, 0.9% sodium chloride solution and 5% glucose solution. The mean value recited in the Table relates to the area between the initial temperature and the fever chart.

Group	Mean Value ± SEM	n
a. Arginine	1.15 ± 0.35	8
b. Ornithine chloride	0.58 ± 0.24	5
c. 0.9% NaCl	2.15 ± 0.24	6
d. 5% glucose	2.22 ± 0.17	5

a/c $p<0.05$
a/d $p<0.05$
a/c $p<0.05$
b/d $p<0.05$

Immobilized arginine in the form of Arginin-Sepharose® (Kabi Pharmacia Fine Chemicals) was used experimentally to bind endotoxins in aqueous solution with the intention of further evaluating the binding ability of the endotoxins with the aid of in vivo and in vitro techniques. Test Examples 3 and 4.

Test Example 3

A small amount of chemically pure glass wool was inserted into each of six Pasteur pipettes to form a column packing. The resultant columns were washed with 6 M hydrochloric acid three times and then with sterilized water and absolute alcohol to obtain a neutral reaction. The columns were then dried at 180°C for four hours in a heated cabinet. 1 ml of Arginin-Sepharose® (gel for affinity chromatography from Kabi Pharmacia Fine Chemicals) to each of these columns. The columns were then washed with 10 total volumes of sterilized water, whereafter 2000 EU of endotoxins from E. Coli were introduced to the columns and allowed to drip therethrough. 1 ml of sterilized water was then introduced into each of said columns, this water also being allowed to drip through the columns. The water that had passed through respective columns was collected (a total of 1.5 ml was collected from the columns, i.e. an amount sufficient to cover the total volume plus the void volume). A physiological saline solution (0.9% sodium chloride solution) was then added to this liquid, so as to obtain a volume of 40 ml. Each of six rabbits was administered intravenously with 10 ml of this mixture for each kilo of body weight and the temperature of the rabbits was recorded once every thirty minutes with the aid of a rectally applied constant-recording analogue temperature probe. Each of six further rabbits were administered intravenously with 10 ml of a physiological sodium chloride solution for each kilo of body weight, said sodium chloride solution being admixed with 500 EU endotoxin per kilo of body weight. The endotoxin was taken from the same batch as that mentioned above. The temperature was measured in the same manner as that aforedescribed. These latter rabbits were used as reference animals (see

Figure 2 and Tables 3a and 3b). A limulus test for endotoxins was carried out on those liquids that had passed through the six Arginin-Sepharose® columns. The solution from all six beds or columns showed a negative result, i.e. the endotoxin concentration did not exceed the detection limit for this system (0.12 EU).

Table 3a

Temperature change in rabbits over a period of 3.0 hours subsequent to injecting endotoxin solution, corresponding to 500 EU/kg body weight which had passed through an Arginine-Sepharose® bed.

A solution of an equivalent amount of endotoxins with an 0.9% saline solution was used as a reference substance.

The mean value disclosed in the Table relates to the area between the line of the initial temperature and the fever chart for n number of observations.

Group	Mean Value ± SEM	n
a. Arginine-Sepharose®	0.34 ± 0.12	6
b. Reference	2.61 ± 0.24	6

a/b $p<0.05$

Table 3b

Maximum rise in the rectal temperature of rabbits subsequent to injecting endotoxin solution corresponding to 500 EU/kg body weight that had passed through an Arginine-Sepharose® bed.

Physiological saline solution in which an equivalent amount of endotoxins (500 EU) had been dissolved was used as a reference substance.

The Table shows the mean value of n number of observations.

Group	Mean Value ± SEM	n
a. Arginine-Sepharose®	0.24 ± 0.05	6
b. Reference	1.33 ± 0.06	6

a/b $p<0.05$

Test Example 4

A separate experiment was carried out in vitro using five different columns which contained endotoxins bound to immobilized arginine in the form of Arginine-Sepharose® (Kabi Pharmacia Fine Chemicals). These columns were prepared in accordance with the aforedescribed Example 3. The columns were washed with sterilized water, whereafter 1400 EU of endotoxins obtained from E. Coli were dripped through the columns. The columns were then eluted with 2 ml of a physiological saline solution (0.9%), whereafter the concentration of endotoxins in the eluate was determined with the aid of a limulus test. This test was chosen because it is an accepted method (Ph. Eur., V. 2.1.9.) and because the method

shows the presence of endotoxins in the solution clearly. The concentration of active endotoxins was measured in the eluate obtained from all five columns and was found to be >110 EU/ml. A further elution was carried out with 2 ml of a 1.8% sodium chloride solution and the endotoxin-concentration of the eluate from all five columns was determined and found to lie within the range of 110-220 EU/ml.

The experiment showed that endotoxins bonded to the Arginine-Sepharose® in the column and that these bonds could be broken by eluting with a saline solution (0.9 or 1.8%).

It is evident from the experiments disclosed in the Test Examples that:

- Among the amino acids tested in Table 1, arginine eliminates the temperature increasing effect of the endotoxins in vivo.

- In the case of constant infusion, arginine and ornithine in vivo are able to eliminate the temperature increasing effect of the endotoxins (Table 2).

- Arginine in an immobilized form has an affinity to and effectively binds endotoxins in vitro (see Tables 3a, 3b and 4).

- Endotoxins are bound to Arginin-Sepharose® and can be eluted therefrom.

The temperature inhibition corresponds to a general endotoxin inhibition, as the immobilizing experiment with Arginine-Sepharose® indicates very clearly. Thus,

freely dissolvable arginine and ornithine, together with structurally-related substances, can be used to eliminate endotoxins in conditions of Gram-negative sepsis with endotoxin shock and ARDS and DIC development. Dosages of 50 mg per kilogram body weight and hour have been find to produce an effect on rabbits. Much higher dosages are required for human use, e.g. dosages of between 5-280 grams per day, suitably under continuous infusion (10-800 mg/kg per hour). (LD_{50} for rats of Arg. Hcl is 3.1 g/kg body weight as a single dosage. Milne, M.D., Pharmacology of Amino Acids. Clinical Pharmacology and Therapeutics, Vol. 9, pages 484-516, 1968). The shock condition is caused by the endotoxins that are produced by the bacteria and not by the bacteria themselves. Endotoxins are present in the blood path even after elimination of the bacteria by the body's own antibacterial system or by means of exogenically administered antibacterial substances. A combined treatment with arginine or structurally-related substances, intravenously/orally in high dosages, and an antibacterial treatment with an appropriate antibiotic is thus clearly indicated.

Under the aforesaid conditions, endotoxins can also be removed by hemofiltration, using a filter which contains immobilized arginine or structurally-related substances. Such filters can also be used to remove pyrogens from distilled water in the manufacture of pharmaceutical preparations intended for intravenous, intramuscular, intracutaneous or intraperitoneal use, and can also be removed from the pharmaceutical preparations themselves. Immobilized arginine or structurally-related substances can also be used to enrich endotoxins for further quantative determination from such solutions as those which are biologically highly active, for example coagulation factor 2 (prothrombin), and factors 8, 9 and 10. The

same method can also be used to remove endotoxins from solutions intended for parenteral use.

Uremia patients who undergo hemodialysis represent a large area in which immobilized arginine or structurally-related substances can be used. These patients relatively often suffer from endotoxin effects, due to the endotoxins penetrating the dialysis filters.

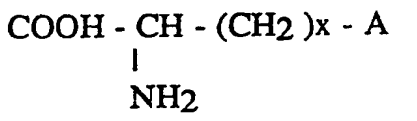
Table 1

Pyrogen reaction in rabbits when testing earlier pyrogen-free amino acids in solutions to which 10 EU endotoxins were added for each 10 ml of solution.

Amino Acid	Conc. (g/l)	Dos. (ml/kg body weight)	Total Temp. Inc. (°C) of three rabbits
Arginine	25	10	0.70 0.65 0.55 0.70
Alanine	20	10	3.40 2.50 2.75
Asparaginic acid	5	10	2.85
Phenyl alanine	25	10	2.25
Glutamic acid	10	10	2.60
Glycine	20	10	2.80 2.65 2.55 2.70
Histidine	15	10	3.60
Isoleucine	20	10	3.40 4.00 2.85 2.00
Leucine	20	10	2.60 2.60 2.65 2.60
Lysine chloride	20	10	1.95 1.60 3.05 2.10
Methionine	10	10	2.05 3.40 1.80 2.95
Proline	10	10	3.15 4.40
Serine	10	10	3.75 3.05 0.45 3.30
Tryptophan	10	10	2.70 3.95 3.50 2.60
Tyrosine	0.5	10	3.25 3.85 2.40 3.20
Threonine	15	10	2.75 4.15 2.35 2.80
Valine	20	10	3.95 3.40 1.70 1.95

CLAIMS

1. Use of a compound according to formula I



(I)

in which x is an integer of from 2 to 5 and

A signifies - NH - C(=NH) - NH_2, - CH_2 - NH_2 or - CO - NH_2

or agmatin, for the preparation of a medicament, which is to be infused in an amount of 10-800 mg/kg body weight and hour for treatment of endotoxin induced effects.

2. The use of a compound according to formula (I) or agmatin for the preparation of a medicament for reducing endotoxin induced fever.

3. The use according to claim 1 or claim 2 **characterized** in that the medicament is administrated in an amount of 10-400 mg/kg body weight and hour.

4. The use according to any of claims 1-3 **characterized** in that the medicament is administred intravenously, intramusculary, intracutaneously or intraperitoneally.

5. The use according to claim 2 **characterized** in that the medicament is administered orally.

6. A method for the treatment of an endotoxin induced effect **characterized** by infusing a compound according to formula (I) or agmatin in an amount corresponding to 10-800 mg per kilogram of body weight and hour, preferably 10-400 mg per kilogram of body weight and hour.

7. A method for the treatment of endotoxin induced fever **characterized** by administering a compound according to formula (I) or agmatin.

8. A method of removing endotoxins from pharmaceutically useful solutions,
pharmaceutical preparations, plasma or blood **characterized** by filtering the pharmaceutically useful solution, the pharmaceutically preparation, the plasma or the blood through a bed which contains an immobilized compound according to formula (I) or immobilized agmatin.

9. A method according to claim 8 **characterized** in that the pharmaceutical preparation contains a biologically active component.

10. A method according to claim 9 **characterized** in that the biologically active comoponent is a coagulation factor.

10. The use of an immobilized compound according to formula (I) or an immobilized agmatin for removing endotoxin from solutions for pharmaceutical use, pharmaceutical preparations, plasma or blood.

11. A method of enriching endotoxins, **characterized** by passing a soluition containing endotoxins through a column with an immobilized compound according to formula (I) or an immobilized agmatin.

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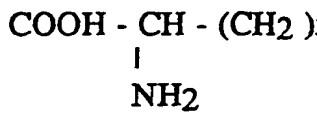
AMENDED CLAIMS

[received by the International Bureau on 1 June 1992 (01.06.92); original claims 10-12 amended; other claims unchanged (2 pages)]

1. Use of a compound according to formula I



(I)

in which x is an integer of from 2 to 5 and

A signifies - NH - C(=NH) - NH_2 or - CH_2 - NH_2

or agmatin, for the preparation of a medicament, which is to be infused in an amount of 10-800 mg/kg body weight and hour for treatment of endotoxin induced effects.

2. The use of a compound according to formula (I) or agmatin for the preparation of a medicament for reducing endotoxin induced fever.

3. The use according to claim 1 or claim 2 **characterized** in that the medicament is administrated in an amount of 10-400 mg/kg body weight and hour.

4. The use according to any of claims 1-3 **characterized** in that the medicament is administred intravenously, intramusculary, intracutaneously or intraperitoneally.

5. The use according to claim 2 **characterized** in that the medicament is administered orally.

6. A method for the treatment of an endotoxin induced effect **characterized** by infusing a compound according to formula (I) or agmatin in an amount corresponding to 10-800 mg per kilogram of body weight and hour, preferably 10-400 mg per kilogram of body weight and hour.

7. A method for the treatment of endotoxin induced fever **characterized** by administering a compound according to formula (I) or agmatin.

8. A method of removing endotoxins from water, pharmaceutically useful solutions, pharmaceutical preparations, plasma or blood **characterized** by filtering the water, the pharmaceutically useful solution, the pharmaceutically preparation, the plasma or the blood through a bed which contains an immobilized compound according to formula (I) or immobilized agmatin.

9. A method according to claim 8 **characterized** in that the pharmaceutical preparation contains a biologically active component.

10. A method according to claim 9 **characterized** in that the biologically active component is a coagulation factor.

11. The use of an immobilized compound according to formula (I) or an immobilized agmatin for removing endotoxin from water, solutions for pharmaceutical use, pharmaceutical preparations, plasma or blood.

12. A method of enriching endotoxins, **characterized** by passing water or a pharmaceutical solution containing endotoxins through a column with an immobilized compound according to formula (I) or an immobilized agmatin.

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Fig 1

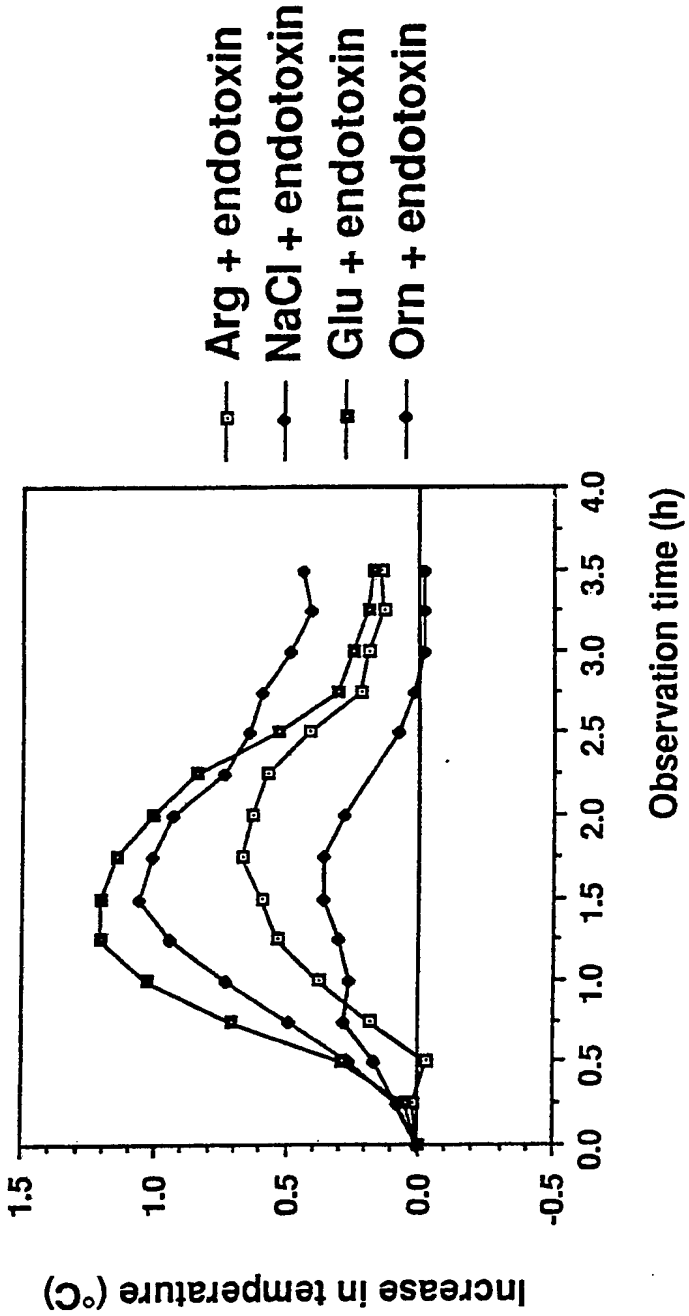
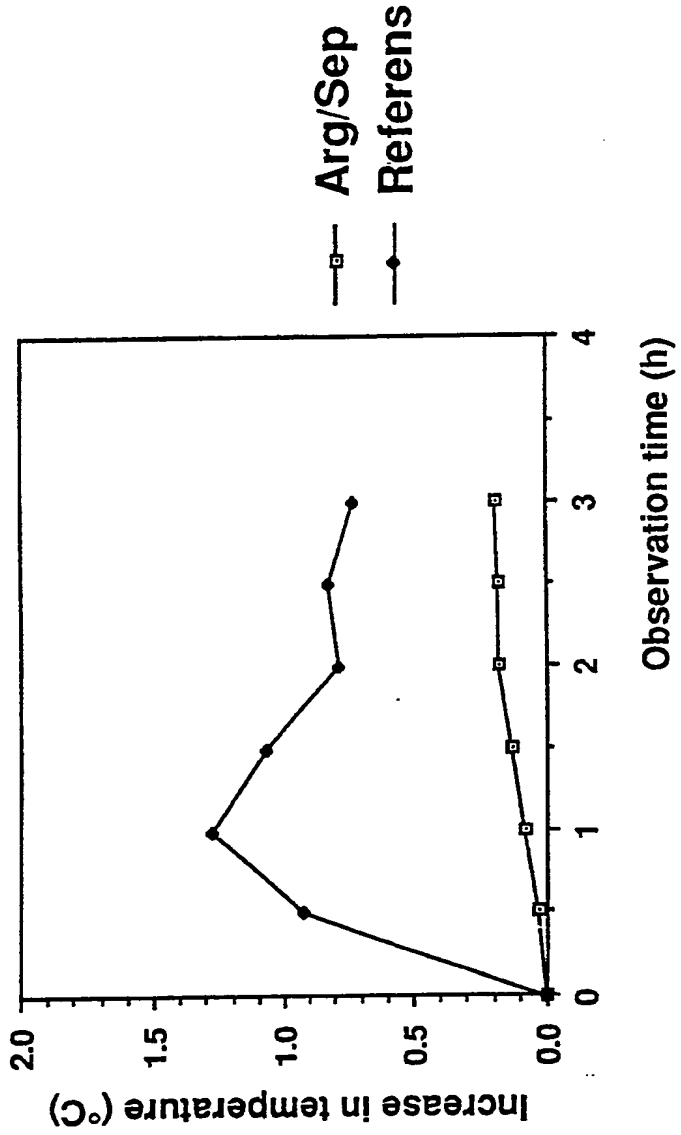

Arg + endotoxin
NaCl + endotoxin
Glu + endotoxin
Orn + endotoxin
Increase in temperature (°C)
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Observation time (h)

Fig 2


Arg/Sep
Referens
Increase in temperature (°C)
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
Observation time (h)

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00893

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 31/155, 31/195, 31/16 // C 07 C 279/14, 279/12
229/26, 237/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	GB, A, 2240041 (SOCIETE DE CONSELLS DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES) 24 July 1991, see the whole document --	1-5
A	US, A, 4308280 (SPORTOLETTI ET AL) 29 December 1981, see column 3, line 38 - column 4, line 39; claim 1 --	1-5
A	US, A, 4405643 (SPORTOLETTI ET AL) 20 September 1983, see column 3, line 39 - column 4, line 38; claim 1 --	1-5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8th April 1992	1992 -04- 13
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Gerd Wranne

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US, A, 4282217 (BAGLIONI ET AL) 4 August 1981, see column 1, line 55 - column 2, line 3 --	1-5
A	EP, A2, 0333474 (MITSUI TOATSU CHEMICALS, INC.) 20 September 1989, see column 2, line 56 - line 63; column 3, line 48 - line 59 column 4, lines 20, 22; column 6, line 21 - line 58; column 7, line 14 - line 44; claims 1-3 --	1-5,8-11
A	STN International, File Medline, STN accession no. 84040139, GL Kovacs et al: "Hormonally active argine -vasopressin suppresses endotoxin-induced fever in rats: lack of effect of oxytocin and a behaviorally active vasopressin fragment", Neuroendocrinology, (1983 Oct) 37 (4) 258-61 --	1-5
A	STN International, File Medline, STN accession no. 88290981, JV Reynolds et al: "Immunomodulatory mechanisms of arginine", Surgery, (1988 Aug) 104 (2) 142-51 --	1-5
A	GB, A, 1253830 (YAMANOUCHI PHARMACEUTICAL CO. LTD) 17 November 1971, see the claims --	1-5
A	EP, A2, 0342139 (SOCIETE CORTIAL S.A.) 15 November 1989, see the claims --	1-5
A	STN International, File Biosis, STN accession no. 91:55734, Biosis accession no. 91:34015, R G Kilbourn et al: "Reversal of endotoxin-mediated shock by N-G methyl-L-arginine an inhibitor of nitric oxide synthesis", Biochem Biophys Res Commun 172 (3), 1990, 1132-1138 --	1-5
I	It is pointed out that a known compound may in many countries be claimed by a product claim restricted to the first medical use and in many of these countries by use claim for other medical indications. (Second medical indication). --	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 6, 7, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00893

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 28/02/92
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A- 2240041	91-07-24	AU-D-	6837690	91-06-27
		DE-A-	4041283	91-06-27
		FR-A-	2656220	91-06-28
		LU-A-	87867	91-05-07
		NL-A-	9002720	91-07-16
		SE-A-	9003974	91-06-23
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		AU-D-	6502880	81-07-02
		BE-A-	886759	81-04-16
		CA-A-	1166160	84-04-24
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		JP-A-	56100717	81-08-12
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4282217	81-08-04	JP-B- 61044844	86-10-04
		LU-A- 83029	81-03-27
		NL-A- 8006707	81-07-16
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